# Instruments (Platform)

# What is the Flow Cell

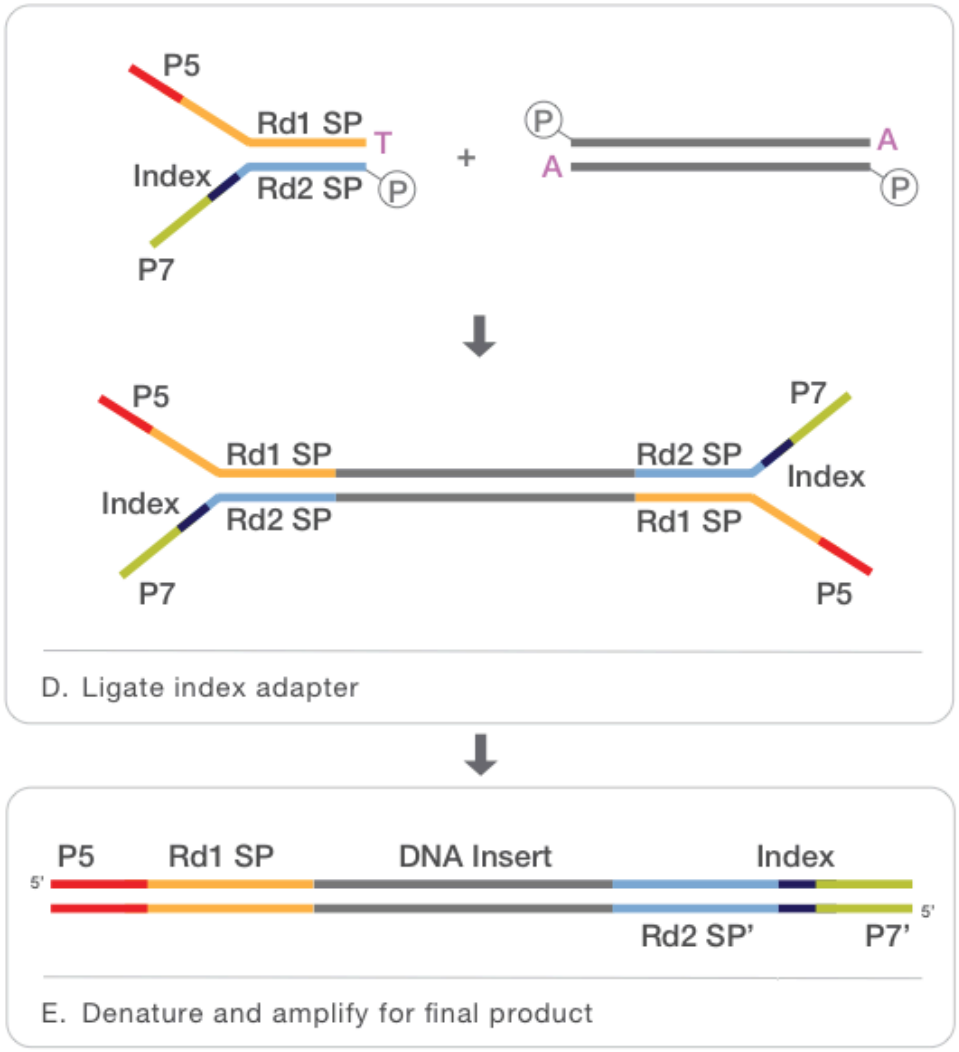
Flow Cell for HiSeq

8 Lane

2 Lane


Flow Cell for MiSeq

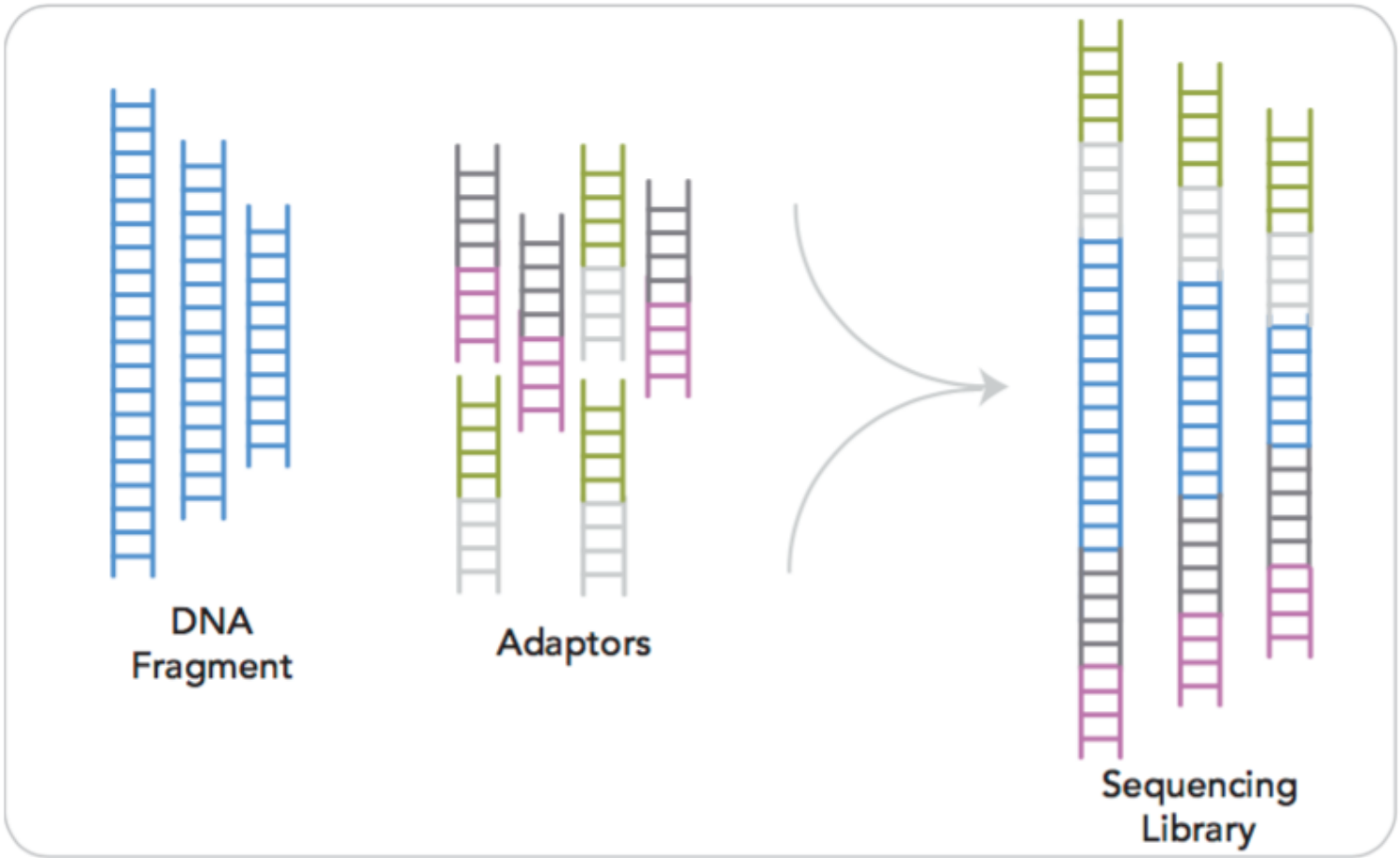
# Library Preparation: Shotgun




- Adapter ligation
  - T-overhangs
  - Forked structure controls orientation
- Library amplification
  - Few cycles
  - Enrich for correctly-adapted fragments
  - Required to complete adapter structure in some protocols
- Size selection
  - Gel excision, AMPure beads
  - Limit insert size as needed, remove artifacts

# What is the adapter?

**Figure 1** Sequencing Library after Paired-End Sample Preparation



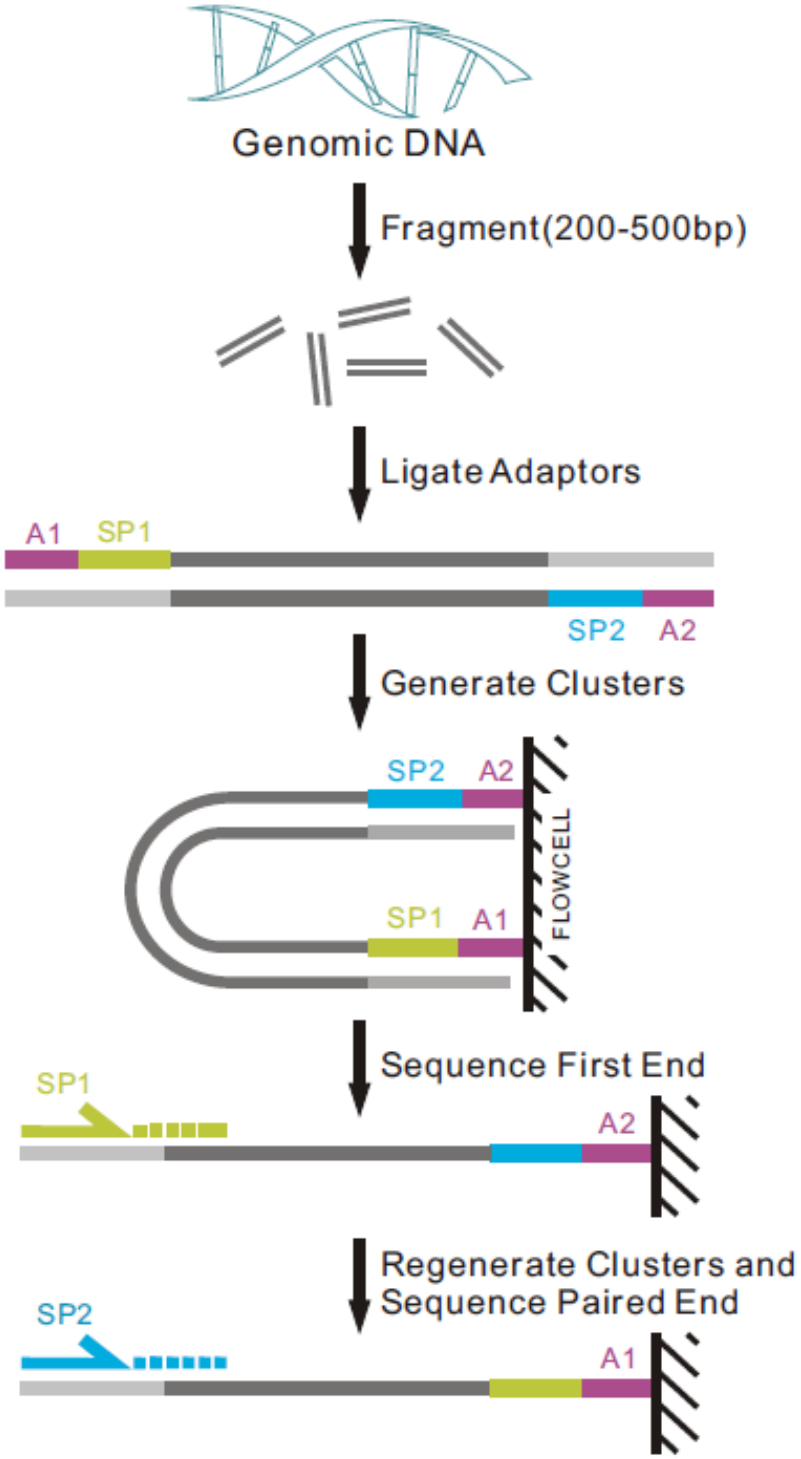
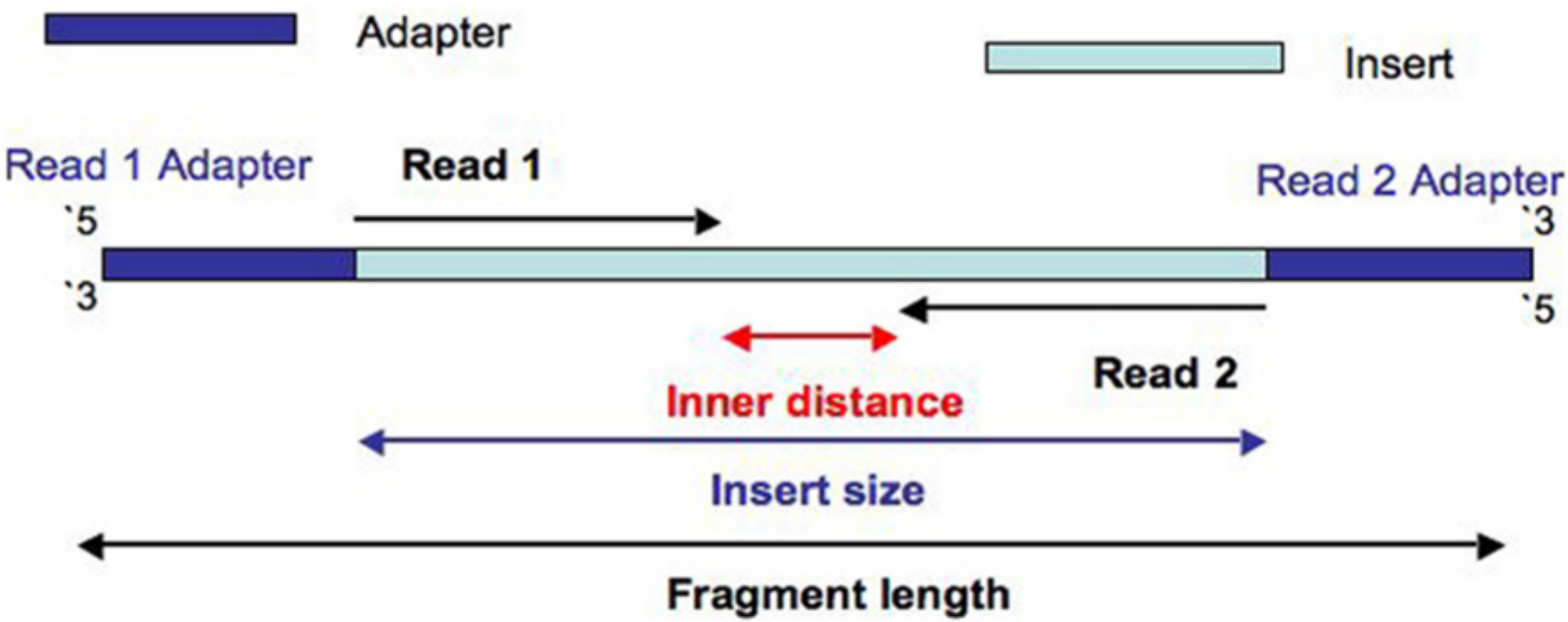



Figure 1-2-1 Pipeline of paired-end sequencing (www.illumina.com)


Adapter
Insert
Read 1 Adapter
Read 1
Read 2 Adapter
`5
`3
`3
`5
Read 2
Inner distance
Insert size
Fragment length

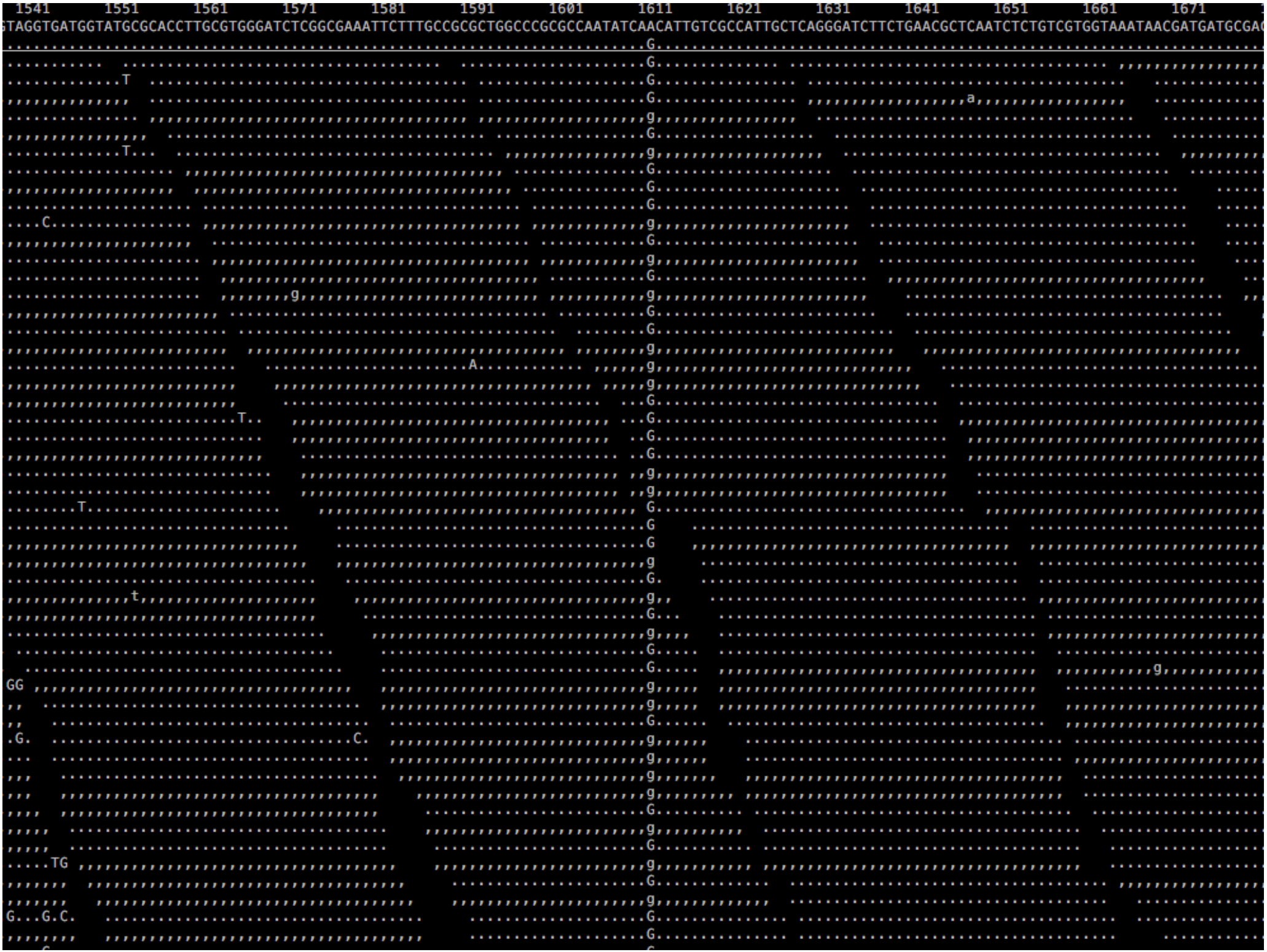
# What is the SAM file?

- SAM stands for Sequence Alignment/Map format

| Col | Field | Type | Regexp/Range | Brief description |
|---|---|---|---|---|
| 1 | QNAME | String | `[!-?A-~]{1,254}` | Query template NAME |
| 2 | FLAG | Int | $[0,2^{16}-1]$ | bitwise FLAG |
| 3 | RNAME | String | `\*|[!-()+-<>-~][!-~]*` | Reference sequence NAME |
| 4 | POS | Int | $[0,2^{31}-1]$ | 1-based leftmost mapping POSition |
| 5 | MAPQ | Int | $[0,2^{8}-1]$ | MAPping Quality |
| 6 | CIGAR | String | `\*|([0-9]+[MIDNSHPX=])+` | CIGAR string |
| 7 | RNEXT | String | `\*|=|[!-()+-<>-~][!-~]*` | Ref. name of the mate/next read |
| 8 | PNEXT | Int | $[0,2^{31}-1]$ | Position of the mate/next read |
| 9 | TLEN | Int | $[-2^{31}+1,2^{31}-1]$ | observed Template LENgth |
| 10 | SEQ | String | `\*|[A-Za-z=.]+` | segment SEQuence |
| 11 | QUAL | String | `[!-~]+` | ASCII of Phred-scaled base QUALity+33 |

# What is the BAM file

- Binary Alignment/Map


1541 1551 1561 1571 1581 1591 1601 1611 1621 1631 1641 1651 1661 1671
GTAGGTGATGGTATGCGCACCTTGCGTGGGATCTCGGCGAAATTCTTTGCCGCGCTGGCCCGCGCCAATATCAACATTGTCGCCATTGCTCAGGGATCTTCTGAACGCTCAATCTCTGTCGTGGTAAATAACGATGATGCGAC